# Postprint: Determination of Crude Protein Digestibility and Available Energy Values of Five Animal Protein Feeds in Raccoon Dogs

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## Abstract

This experiment aimed to determine the digestibility of crude protein and effective energy values in raccoon dogs fed five commonly used animal protein feedstuffs. Thirty healthy male and thirty female raccoon dogs with similar body weight were selected and randomly divided into six groups, with ten animals per group (half male and half female), one animal per replicate, and housed individually in cages. The six groups of raccoon dogs were fed either a basal diet or test diets in which the basal diet was replaced by 20% (fish meal, chicken meal, or meat and bone meal) or 15% (blood meal or enzymatically hydrolyzed feather meal) of the feedstuffs to be evaluated. The experiment consisted of a 7-day preliminary period followed by a 5-day formal collection period. The results showed that there were no significant differences ($P>0.05$) between male and female raccoon dogs in the digestibility of dry matter, organic matter, crude protein, digestible energy, and metabolizable energy of the five animal protein feedstuffs. The digestible energy values for raccoon dogs fed fish meal, meat and bone meal, chicken meal, blood meal, and enzymatically hydrolyzed feather meal were 17.30, 12.60, 17.09, 20.27, and 18.84 MJ/kg, respectively; the metabolizable energy values were 13.94, 10.38, 14.44, 16.05, and 15.10 MJ/kg, respectively; and the crude protein digestibility values were 94.8%, 80.7%, 81.0%, 92.6%, and 87.5%, respectively. It was concluded that, as protein feed resources, fish meal had the highest crude protein digestibility in raccoon dogs, followed by blood meal and enzymatically hydrolyzed feather meal, while chicken meal and meat and bone meal had relatively lower crude protein digestibility.

## Full Text

# Determination of Crude Protein Digestibility and Effective Energy Values of Five Animal Protein Feeds for Raccoon Dogs

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### Abstract

This experiment was conducted to determine the crude protein digestibility and effective energy values of five commonly used animal protein feeds for raccoon dogs. Sixty healthy raccoon dogs (30 males and 30 females) with similar body weight were selected and randomly divided into 6 groups, with 10 replicates per group (half male and half female) and one raccoon dog per replicate, housed individually in cages. The six groups were fed either a basal diet or experimental diets in which 20% of the basal diet was replaced by fish meal, chicken meat meal, or meat and bone meal, or 15% was replaced by blood meal or enzyme-hydrolyzed feather meal. The experiment consisted of a 7-day adaptation period followed by a 5-day collection period. The results showed no significant differences between male and female raccoon dogs in the digestibility of dry matter, organic matter, crude protein, digestible energy (DE), or metabolizable energy (ME) for any of the five animal protein feeds (P>0.05). The DE values of fish meal, meat and bone meal, chicken meat meal, blood meal, and enzyme-hydrolyzed feather meal for raccoon dogs were 17.30, 12.60, 17.09, 20.27, and

18.84 MJ/kg, respectively; the ME values were 13.94, 10.38, 14.44, 16.05, and 15.10 MJ/kg, respectively; and the crude protein digestibility values were 94.8%, 80.7%, 81.0%, 92.6%, and 87.5%, respectively. These results indicate that, as protein feed resources for raccoon dogs, fish meal has the highest crude protein digestibility, followed by blood meal and enzyme-hydrolyzed feather meal, while chicken meat meal and meat and bone meal have relatively lower crude protein digestibility.

**Keywords:** animal protein feed; crude protein digestibility; digestible energy; metabolizable energy; raccoon dogs

## Introduction

Animal protein feeds are important sources of protein in animal diets. Determining the digestibility of energy and nutrients in these feeds is essential for formulating scientifically rational diets. Previous studies have evaluated the digestibility of dry matter, crude protein, digestible energy, and metabolizable energy of fish meal in pigs [1-2], chickens [3-4], mink [5], geese [6-7], and dogs [8]; chicken meat meal in pigs [9] and blue foxes [10]; meat and bone meal in pigs [11] and blue foxes [10]; blood meal in mink [5] and geese [7]; and enzyme-hydrolyzed feather meal in pigs [12], blue foxes [10], and mink [5]. However, no studies have been reported on the nutritional value of fish meal, chicken meat meal, meat and bone meal, blood meal, or enzyme-hydrolyzed feather meal for raccoon dogs. Therefore, this experiment was conducted to evaluate the nutritional value of these five animal protein feeds for raccoon dogs and to provide a basis for rational diet formulation.

## Materials and Methods

### Experimental Animals and Diets

This experiment was conducted using 60 raccoon dogs (30 males and 30 females) at 11 weeks of age with similar body weight, purchased on September 1, 2016. The animals were randomly divided into 6 groups, with 10 raccoon dogs per group (half male and half female), one raccoon dog per replicate, and housed individually in cages.

The nutrient levels of the five animal protein feeds are shown in Table 1 . A basal diet containing 22% crude protein was formulated according to the enterprise standards of Hebei Qiuhao Group Huaxia Xinnong Technology Co., Ltd. (composition and nutrient levels of the basal diet are shown in Table 2 ). Considering the typical usage levels and palatability of the five animal protein feeds in raccoon dog diets, test diets were formulated by replacing 20% of the basal diet

with fish meal, meat and bone meal, or chicken meat meal, or by replacing 15% with blood meal or enzyme-hydrolyzed feather meal [13].

The experimental diets were provided as powdered air-dried feed. Based on the principle of 90% ad libitum intake, 200 g of diet was weighed daily and mixed with water at a 1:3 mass ratio to form a paste before feeding. Raccoon dogs were fed twice daily at 08:00 and 15:00. They had free access to drinking water, were exposed to natural lighting, and cages were cleaned regularly.

The adaptation period was from September 10 to September 16, 2016, and the formal trial began on September 17, 2016. At the start of the collection period, male raccoon dogs weighed 5.33 ± 0.21 kg and females weighed 5.28 ± 0.17 kg. Feces and urine were collected for 5 days using the total collection method.

### Sample Collection and Preparation

Before the experiment began, hydrochloric acid was added to the urine buckets for nitrogen preservation (20 mL of 10% HCl added daily). Urine volume was measured with a graduated cylinder, and a 1/20 aliquot of the 5-day urine collection was sampled for determination of gross energy. Feces were collected daily and weighed, and 10 mL of 10% HCl solution was added per 100 g of fresh feces for nitrogen fixation. Fresh feces were first sterilized at 80°C for 2 h, then dried to constant weight at 65°C to prepare air-dried samples. The dried fecal samples were ground to pass through a 40-mesh sieve to produce analytical samples for determination of gross energy and nutrient content.

### Measurements and Calculations

The gross energy in diets, feces, and urine samples was determined using an IKA C2000 basic oxygen bomb calorimeter (Staufen, Germany). Dry matter content was determined according to GB/T 6435-2014, crude protein content by the Kjeldahl method according to GB/T 6432-1994, crude fat content by Soxhlet extraction according to GB/T 6433-2006, and crude ash content according to GB/T 6438-2007. Organic matter content was calculated as original weight minus crude ash weight. Calcium, iron, potassium, magnesium, copper, zinc, and manganese contents in test feeds and diets were determined using an IRIS Intrepid II plasma emission spectrometer (TE, USA) according to GB/T 13885-1992. Phosphorus content in raw materials and diets was determined by spectrophotometry according to GB/T 6437-2002. National standard material bovine liver powder [GBW(E) 080193] was used as a quality control standard. Amino acid contents (except tryptophan) in raw materials and diets were determined using an amino acid automatic analyzer (Hitachi L-8900, Tokyo, Japan) according to GB/T 18246-2000. Tryptophan content was determined by spectrophotometry using a 754PG UV-Vis spectrophotometer (Shanghai Optical Instrument Co., Ltd.) according to GB/T 15400-94.

The digestibility of nutrients or gross energy in the test feeds was calculated using the substitution method with the following formula:

$D = [100 \times (A - B)/F] + B$ [13]

Where: $D$ is the digestibility (%) of a nutrient or gross energy in the test feed; $A$ is the digestibility (%) of that nutrient or gross energy in the test diet; $B$ is the digestibility (%) of that nutrient or gross energy in the basal diet; and $F$ is the proportion of that nutrient in the test feed relative to the test diet.

The digestibility of nutrients or gross energy, gross energy utilization, digestible energy, and metabolizable energy in test diets and basal diets were calculated as follows:

Nutrient or gross energy digestibility (%) = (intake of nutrient or gross energy - fecal excretion of nutrient or gross energy) × 100 / intake of nutrient or gross energy;

Gross energy utilization (%) = (gross energy intake - fecal energy - urinary energy) × 100 / gross energy intake;

Digestible energy (MJ/kg) = gross energy × gross energy digestibility / 100;

Metabolizable energy (MJ/kg) = gross energy × gross energy utilization / 100.

### Statistical Analysis

Results are expressed as mean ± standard deviation. The t-test in SAS 6.12 was used to examine significant differences between male and female raccoon dogs. The ANOVA procedure was used to analyze variance in nutrient or gross energy digestibility, DE, and ME among different test feeds. When ANOVA showed significant differences, the LSD method was used for pairwise comparison of means. $P<0.05$ was used as the criterion for statistical significance.

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## Results

### Energy Digestibility and Effective Energy Values

As shown in Table 3 , raccoon dog sex had no significant effect on DE or ME ($P>0.05$). The gross energy digestibility of fish meal and blood meal was relatively high at 93.7% and 91.6%, respectively, significantly higher than the other three test feeds ($P<0.05$). The gross energy digestibility of enzyme-hydrolyzed feather meal and chicken meat meal was intermediate at 84.8% and 81.5%, respectively, while meat and bone meal had the lowest gross energy digestibility at 79.5%. The DE of blood meal was 20.27 MJ/kg, significantly higher than the other four test feeds ($P<0.05$), followed by enzyme-hydrolyzed feather meal at 18.84 MJ/kg. The DE values of fish meal and chicken meat meal were similar at 17.30 and 17.09 MJ/kg, respectively. Meat and bone meal had the lowest DE at 12.60 MJ/kg, significantly lower than the other four test feeds. The gross energy utilization of fish meal was 75.5%, significantly higher than the

other four test feeds (P<0.05). Meat and bone meal had the lowest gross energy utilization at only 65.4%, significantly lower than fish meal and blood meal (P<0.05). The gross energy utilization of chicken meat meal, blood meal, and enzyme-hydrolyzed feather meal ranged from 68.0% to 72.5%. The ME values of blood meal and enzyme-hydrolyzed feather meal were 16.05 and 15.10 MJ/kg, respectively, significantly higher than the other three test feeds (P<0.05). The ME values of fish meal and chicken meat meal were similar at 13.94 and 14.44 MJ/kg, respectively, while meat and bone meal had the lowest ME at 10.38 MJ/kg.

### Digestibility of Dry Matter, Organic Matter, and Crude Protein

As shown in Tables 4 , 5 , and 6 , there were no significant differences between male and female raccoon dogs in the digestibility of dry matter, organic matter, or crude protein for any of the five animal protein feeds (P>0.05).

As shown in Table 4, blood meal had the highest dry matter digestibility at 86.2%, which was not significantly different from enzyme-hydrolyzed feather meal (P>0.05) but was significantly higher than the other three test feeds (P<0.05). Meat and bone meal had the lowest dry matter digestibility at only 53.9%, significantly lower than the other four test feeds (P<0.05).

As shown in Table 5, fish meal had the highest organic matter digestibility at 92.3%, which was not significantly different from blood meal (P>0.05) but was significantly higher than the other three test feeds (P<0.05). Enzyme-hydrolyzed feather meal was intermediate at 54.8%; meat and bone meal and chicken meat meal had lower organic matter digestibility at 80.5% and 80.9%, respectively, significantly lower than the other three test feeds (P<0.05).

As shown in Table 6, the crude protein digestibility of fish meal and blood meal was 94.8% and 92.6%, respectively, significantly higher than the other three test feeds (P<0.05). The crude protein digestibility of enzyme-hydrolyzed feather meal was 87.5%, which was intermediate. Meat and bone meal and chicken meat meal had lower crude protein digestibility at 80.7% and 81.0%, respectively.

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## Discussion

### Digestive Utilization of Fish Meal Nutrients and Energy

Due to the lack of evaluated nutritional data for commonly used feeds for raccoon dogs, diet formulation for raccoon dogs often relies on experimental data from pigs or other fur-bearing animals such as foxes and mink. In the Chinese Feed Composition and Nutritional Value Table (2017 28th Edition), the DE and ME values of imported fish meal (containing 67.0% crude protein) for pigs are 13.47 and 11.16 MJ/kg, respectively. Rojas et al. [2] reported that herring fish meal (containing 63.4% crude protein with 18.51 MJ/kg gross energy) had

a gross energy digestibility of 86.3%, DE of 15.89 MJ/kg, ME of 14.53 MJ/kg, and protein digestibility of 84.1% in pigs. Sun Weili et al. [10] reported that when imported fish meal (containing 68.22% crude protein) was used as the sole protein source in a purified diet, adult male and female blue foxes had dry matter and crude protein digestibility values of 64.43% and 60.18%, respectively, which were not significantly different from a feather meal diet. Fan Wenwen et al. [5] used the substitution method with 20% fish meal (containing 65.8% crude protein with 22.56 MJ/kg gross energy) replacing the basal diet and found that black female mink had gross energy digestibility of 83.5%, dry matter digestibility of 67.1%, and crude protein digestibility of 89.5% for fish meal. In the present experiment, the dry matter digestibility, crude protein digestibility, and DE of fish meal for raccoon dogs were all higher than those reported in the above studies. Two reasons may account for these differences: first, different diet palatability and amino acid balance may have affected nutrient digestibility [10]; second, using data from pigs or other fur-bearing animals for calculating raccoon dog dry powder diets may lead to deviations. Among the five animal protein feeds tested, the dry matter digestibility of fish meal was lower than that of blood meal, which may be related to the higher crude ash content in the fish meal diet. However, the organic matter, gross energy, and crude protein digestibility of fish meal were all higher than those of the other test feeds, further demonstrating the high nutritional value of fish meal for raccoon dogs.

**Digestive Utilization of Meat and Bone Meal Nutrients and Energy**

In the Chinese Feed Composition and Nutritional Value Table (2017 28th Edition), the DE and ME values of meat and bone meal (containing 50.0% crude protein) for pigs are 11.84 and 10.17 MJ/kg, respectively. Shi et al. [14] reported that growing pigs had gross energy digestibility and utilization rates of 75.6% and 73.1%, respectively, and DE and ME values of 13.96 and 13.49 MJ/kg for meat and bone meal (containing 56.4% crude protein with 18.46 MJ/kg gross energy). Sows had gross energy digestibility and utilization rates of 84.6% and 78.2%, respectively, and DE and ME values of 15.62 and 14.43 MJ/kg for meat and bone meal. Sun Weili et al. [10] reported that when meat and bone meal (containing 51.5% crude protein) was used as the sole protein source in a purified diet, adult blue foxes had dry matter digestibility of 49.7% and crude protein digestibility of 54.0% for the meat and bone meal diet, with dry matter digestibility significantly lower than feather meal, fish meal, and chicken meat meal diets, and crude protein digestibility numerically lower than these diets as well. In the present experiment, the DE and ME of meat and bone meal for raccoon dogs were slightly higher than the corresponding values for pigs in the Chinese Feed Composition and Nutritional Value Table (2017 28th Edition), but the gross energy digestibility was similar to that of pigs. The relatively low gross energy utilization of meat and bone meal by raccoon dogs may be related to the fact that while pigs primarily use protein for weight gain, raccoon dogs also use protein for hair follicle development. In this experiment, the dry matter and crude protein digestibility of meat and bone meal for raccoon dogs were

higher than those for blue foxes, but like blue foxes, the dry matter digestibility of meat and bone meal was lower than that of other test feeds, indicating that the high crude ash content in meat and bone meal diets cannot be completely digested and absorbed.

### Digestive Utilization of Chicken Meat Meal Nutrients and Energy

Rojas et al. [9] used corn and chicken meat meal (containing 96.80% dry matter, 66.0% crude protein, and 20.53 MJ/kg gross energy) to formulate weaned piglet diets and measured gross energy digestibility of 87.9% and DE and ME values of 17.41 and 15.46 MJ/kg for chicken meat meal. Sun Weili et al. [10] reported that when chicken meat meal (containing 52.3% crude protein) was used as the sole protein source in a purified diet, adult blue foxes had dry matter and crude protein digestibility values of 64.4% and 63.9%, respectively, for the chicken meat meal diet, with male foxes showing significantly higher dry matter and crude protein digestibility than meat and bone meal and pork meal diets. In the present experiment, the gross energy digestibility, DE, and ME of chicken meat meal for raccoon dogs were slightly lower than those for weaned piglets [9], while dry matter and crude protein digestibility were higher than those for blue foxes [10]. However, unlike blue foxes, no significant differences were observed between chicken meat meal and meat and bone meal in organic matter and crude protein digestibility for raccoon dogs, indicating that raccoon dogs have similar digestive capacity for protein from poultry and livestock meat meals.

### Digestive Utilization of Blood Meal Nutrients and Energy

In the Chinese Feed Composition and Nutritional Value Table (2017 28th Edition), the DE and ME values of blood meal (containing 82.8% crude protein) for pigs are 11.42 and 9.04 MJ/kg, respectively. Deng Yingying et al. [15] reported that weaned piglets had crude protein digestibility of 92.7%, gross energy digestibility and utilization rates of 92.0% and 90.7%, respectively, and DE and ME values of 19.28 and 19.03 MJ/kg for spray-dried broken-cell blood globin powder (containing 91.9% dry matter and 90.1% crude protein). Fan Wenwen et al. [5] reported that mink had dry matter digestibility of 84.9% for porcine blood globin powder (containing 91.6% dry matter and 92.3% crude protein), which was significantly higher than imported fish meal; crude protein and gross energy digestibility were 95.9% and 92.2%, respectively, not significantly different from imported fish meal. In the present experiment, the dry matter, crude protein, and gross energy digestibility of blood meal for raccoon dogs were similar to those of the spray-dried broken-cell blood globin powder [15] and porcine blood globin powder [5] mentioned above. Moreover, the DE and ME of blood meal for raccoon dogs were much higher than the DE and ME values of blood meal for pigs in the Chinese Feed Composition and Nutritional Value Table (2017 28th Edition), and the crude protein digestibility was similar to that of imported fish meal, indicating that blood meal can serve as a high-quality protein feed resource for raccoon dogs.

**Digestive Utilization of Enzyme-Hydrolyzed Feather Meal Nutrients and Energy**

In the Chinese Feed Composition and Nutritional Value Table (2017 28th Edition), the DE and ME values of feather meal (containing 77.9% crude protein) for pigs are 11.59 and 9.29 MJ/kg, respectively. Yu Yang [12] used 5% enzyme-hydrolyzed feather meal (containing 85.5% crude protein) to replace the basal diet and measured crude protein digestibility of 83.8% for growing-finishing pigs. Sun Weili et al. [10] used feather meal (containing 85.72% crude protein) as the sole protein source in a purified diet and determined dry matter and crude protein digestibility values of 68.57% and 61.96%, respectively, for adult blue foxes, which were not significantly different from imported fish meal and chicken meat meal diets but were significantly higher than meat and bone meal and pork meal diets for dry matter digestibility, and crude protein digestibility was also significantly higher than the pork meal diet. Fan Wenwen et al. [5] used 20% feather meal (containing 87.63% crude protein with 25.07 MJ/kg gross energy) to replace the basal diet and found that black female mink had dry matter digestibility of 40.63%, gross energy digestibility of 47.36%, and crude protein digestibility of 51.10% for feather meal, all significantly lower than imported fish meal. In the present experiment, the crude protein digestibility of enzyme-hydrolyzed feather meal for raccoon dogs was similar to that for growing-finishing pigs, but its gross energy, dry matter, and crude protein digestibility were all higher than those for blue foxes and mink. Additionally, the DE and ME were higher than the corresponding values for feather meal for pigs in the Chinese Feed Composition and Nutritional Value Table (2017 28th Edition). The enzyme-hydrolysis process breaks disulfide bonds in feathers and thereby improves feather meal digestibility, which has been confirmed [16]. Whether differences exist among the three fur-bearing animals in the digestive utilization of enzyme-hydrolyzed feather meal requires further research.

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## Conclusions

1. Among the five animal protein feeds, fish meal had the highest gross energy and crude protein digestibility, making it a high-quality protein feed resource for formulating dry powder diets for raccoon dogs.
2. Provided that palatability and amino acid balance are satisfied, blood meal, enzyme-hydrolyzed feather meal, and chicken meat meal can be used appropriately in raccoon dog dry powder diets.
3. Meat and bone meal has high crude ash content and is not suitable for large-scale use in raccoon dog dry powder diets.

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## Tables

**Table 1** Nutrient levels of five animal protein feeds (air-dry basis)

**Table 2** Composition and nutrient levels of the basal diet (air-dry basis)

**Table 3** GE digestibility, DE and ME of five animal protein feeds for raccoon dogs

**Table 4** DM digestibility of five animal protein feeds for raccoon dogs

**Table 5** OM digestibility of five animal protein feeds for raccoon dogs

**Table 6** CP digestibility of five animal protein feeds for raccoon dogs

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*Note: Figure translations are in progress. See original paper for figures.*